ETHANOLIC EXTRACTS FROM BLACK (*PIPER NIGRUM* L.) AND CUBEB PEPPER (*PIPER CUBEBA* L.) FRUITS AVAILABLE IN SERBIA: COMPARATIVE STUDY REGARDING PHYTOCHEMICAL PROPERTIES, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES

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The present study aimed to compare phytochemical properties (total extractive matter, phenolic, flavonoids, and mineral content), antimicrobial and antioxidant activities of black pepper fruits' ethanolic extract (BPFEE), and cubeb pepper fruits' ethanolic extract (CPFEE). The extracts were prepared by reflux extraction under identical conditions (solvomodule 1:10 m/v, 2 h at boiling point). BPFEE had higher total phenolic and flavonoids content, lower total extractive matter and better antioxidant activity than CPFEE. Among the macroelements, the highest presence of Na was detected in both extracts in similar amounts. BPFEE contained higher amounts of K, Ca, P and Mg. Amongst the microelements, Cu and Li were detected in both extracts in similar amounts. However, BPFEE contained higher amounts of Zn, but lower amount of Fe, compared to CPFEE. The antimicrobial activity was tested against *Bacillus cereus, Salmonella* spp. and *Candida albicans*, pairs of reference strains and isolates. CPFEE resulted as a better bacteriostatic and anticandidal agent. These results are valuable in promoting further progress, development and production regarding the aromatic plant industry, pharmaceutical and food industry.

Keywords: Antimicrobial; Black pepper (*Piper nigrum* L.); Cubeb pepper (*Piper cubeba* L.); Extracts; Mineral content; isolation; phenolic compounds; Soxhlet extraction

Introduction

Two most cultivated and used species from Piperaceae family are black pepper (*Piper nigrum* L.) and cubeb pepper (*Piper cubeba* L.), highly appreciated spices and medicinal plants. Globally, black pepper is unavoidable as a spice and natural preservative during food preparation, including in Serbian folk cuisine. Since it is not grown in the area of the Balkan Peninsula, black pepper is mostly imported from Vietnam. Cubeb pepper arrived in Europe via India and is used mostly in western European countries as a spice and flavouring agent; however, its use in Serbia and other Balkan countries is quite rare.

The biological effects of these peppers are numerous. For example, black pepper is reported to possess antimicrobial, antioxidant, anti-inflammatory, analgesic, antidiabetic, hepato- and neuroprotective activities [1], while cubeb pepper is considered a potent carminative, diuretic, expectorant, antiseptic and antimicrobial agent in folk medicine [2,3].

Both black and cubeb pepper fruits contain various biologically active compounds, such as glycosides, alkaloids, phenols, flavonones, tannins and saponins [4,5]. Besides fibers, proteins, vitamins and bioactive compounds, these peppers are good sources of minerals, such as Ca, Mg, Fe, Mn, Zn, K, Mg and P [3,6]. Aqueous extract of cubeb pepper showed high levels of P and Mg [7]. According to Fatima et al. [8] cubeb pepper had the highest content of K, Fe and Na, suggesting that intake of approximately 16 g of cubeb berry provides the daily need of Fe in human nutrition.

Even today, the spread of pathogenic and treatment-resistant microorganisms is considered a massive global health concern. For example, Gram-positive *Bacillus* strains cause food poisoning and serious infections of different organs [9,10]. Gram-negative *Salmonella* spp. is a foodborne pathogen, commonly found in the intestinal tract of domestic or wild animals. It is transmitted through fecal contamination of food and other products, causing infections that can be challenging to treat [11]. *Candida albicans* as an opportunistic pathogenic yeast is especially dangerous for people with weakened immune system, even resulting in systemic candidiasis [12]. Thus, the research regarding the design of both synthetic and

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natural-based products with wide spectrum of antimicrobial activities against pathogenic microorganisms is constantly occurring.

The use of „green“, safe and ecologically acceptable solvents for obtaining extracts from natural sources is particularly necessary for the production of pharmaceutical, nutritional and cosmetic formulations. Despite defined as an ideal „green“ solvent, water is not efficient for extracting non-polar and semi-polar compounds [13]. Although methanol is an adequate substitution, ethanol is less toxic, cheap, environmentally-friendlier solvent, commonly included in numerous medical formulations, as well as obtained from agricultural feedstocks containing starch and cellulose [14,15]. After extraction, leftover solid residues from plant material (containing insoluble cellulosic compounds) can be revalorized as biomass precursors for production of energy, bio-ethanol, biological derivatives and as an additive in animal feed [16], leading to successful industrialization of the technological process and maximizing the profit of the used plant material.

The aim of the present study is preparation of black pepper fruits' ethanolic extract (BPFEE) and cubeb pepper fruits' ethanolic extract (CPFEE), comparison of their phytochemical properties (total extractive matter, phenolic, flavonoids and mineral content), antimicrobial and antioxidant activities. Ethanol was used as a solvent due to its non-toxicity and good yield of extracts, while reflux extraction was chosen over other methods because of its shorter extraction time, as confirmed in the literature [17-19].

Materials and methods

Plant material

Commercial samples of black pepper fruits ("Solunac Gyros and top spices", Ugrinovački put, part 25-No.32, Zemun, Serbia; country of origin: Vietnam) and cubeb pepper fruits (Spice Up, Čumićeva bb, Belgrade, Serbia; country of origin: Indonesia, imported from Austria) were used. The materials were refrigerated in original packages and protected from light. A laboratory electric mill (Braun Aromatic KSM2) was used for grinding the plant materials before the analysis. Granulometric analysis found that black pepper and cubeb pepper had mean particle diameters of 0.258 mm and 0.360 mm, respectively. Gravimetric analysis determined the moisture content in the plant material, which was 10.67% of the sample for black pepper and 9.56% of the sample for cubeb pepper.

Reagents and chemicals

Ethanol (96%), anhydrous Na_2CO_3 (99%), $AlCl_3 \cdot 6H_2O$ (99%) and concentrated HNO_3 (65%) were purchased from Zorka-Pharma (Šabac, Serbia). Gallic acid (97%) and rutin (pro analysis grade) were from Merck WGK (Darmstadt, Germany). Dimethyl sulfoxide (DMSO) (≥99%), butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picrylhydrazyl (DPPH radical), Folin-Ciocalteu reagent and CH_3COOK (pro analysis grade) were provided from Sigma Aldrich (Munich, Germany). Triphenyltetrazolium chloride (TTC) was from Sigma Chemical Company (St. Louis, USA). Purified distilled water (HPLC grade, Fisher Chemical), Multistandard IV (multielement standard solution, Merck, concentration of 1000 ppm) and Argon 5.0 (99.999% purity) as the carrier gas were used for ICP-OES analysis.

Preparation of ethanolic extracts

The grounded (black or cubeb) pepper fruits (2.5 g) were mixed with ethanol (solvomodule 1:10 m/v) and subjected to reflux extraction for 2 h at boiling point. After extraction, the plant material and extracts were separated by vacuum filtration. Excess ethanol was removed by evaporation on a rotary evaporator at 50 °C, followed by drying the extracts in a vacuum oven at 40 °C until constant weight. The products were refrigerated at 4 °C.

Phytochemical analysis

Determination of total extractive matter (TEM), total phenolic content (TPC) and total flavonoids content (TFC)

By drying the extract at 105 °C to constant weight, TEM was determined gravimetrically and expressed as grams per 100 grams of plant material (g/100 g p.m.).

A modified Folin-Ciocalteu method [20] was used to determine TPC spectrophotometrically. Gallic acid was used as a standard (the concentration range was 0.00625-0.2 mg/cm^3). After diluting 0.5 cm^3 of ethanolic extract with 4.5 cm^3 distilled water, 0.5 cm^3 of Folin-Ciocalteu reagent and 5 cm^3 Na_2CO_3 were added. After 1.5 h of incubating the mixture at room temperature, the absorbance was determined at 765 nm. The results were derived from a calibration curve ($A_{765nm} = 0.01582 + 5.04832 \cdot c_{GA}$, $R^2 = 0.9991$) and expressed as gallic acid equivalents per gram of dry extract (mgGAE/g d.e.).

TFC was determined by the aluminium chloride colorimetric method, where Al (III) was used as a complexing agent [21], with certain modifications. Rutine was used as a standard (the concentration range was 0.005-0.1 mg/cm^3). Generally, 2 cm^3 of ethanolic extract was mixed with 0.1 cm^3 of 10% $AlCl_3 \cdot 6H_2O$, 0.1 cm^3 of 1 M CH_3COOK and 2.8 cm^3 of distilled water. After 40 min of incubation at room temperature, the absorbance was determined at 415 nm. The results were derived from a calibration curve ($A_{415} = 14.171 \cdot c_R + 4.61 \cdot 10^{-2}$, $R^2 = 0.9991$) and expressed as rutine equivalents per gram of dry extract (mgRE/g d.e.).

To calculate the standard deviation, all experiments were repeated three times.

Determination of elements by ICP-OES

The standard calibration solutions were prepared

by diluting the Multistandard IV to ensure that the concentrations of the standards for calibration diagrams were in the expected range of the tested elements. Calibration parameters are listed in Table 1. Wet digestion was used to prepare the extract samples: 0.2 cm^3 of selected extract was mixed with 1 cm^3 of concentrated HNO_3 (65%) and heated in a water bath until the solution was discolored and the evaporation of steam ceased. The purified distilled water was used to dilute the samples after they were filtered through a 0.45 μm Millipore filter. Quantitative analysis was performed on ICP-OES apparatus (ARCOS FHE12, SPECTRO, Germany) at the operating conditions and parameters provided in Table 1.

Table 1. Calibration parameters and operating conditions for ICP-OES

Element	Detection wavelength, nm	Correlation coefficient (R^2)	Limit of detection, μg/dm^3	Linearity range, mg/dm^3	Operating conditions for ICP-OES	
As	197.2	0.999	2.55	0.002–3.6	Plasma power (W)	1400
					Gas flow (L/min)	
Ag	224.6; 328.1	0.999; 0.999	0.39	0.014–12; $3\cdot10^{-4}$–12	-Coolant	13
					-Auxiliary	0.80
B	182.6; 249.7	0.999; 0.999	6.43	0.007–12; 0.006–12	Nebulizer type	Cross flow
Ba	233.5	0.995	0.183	$1.83\cdot10^{-4}$–12	Nebulizer flow rate (L/min)	0.95
Bi	190.2; 223.1	0.999; 0.999	3.53	0.003–12; 0.004–12	Pump speed	30
Ca	183.8; 396.8	0.999; 0.999	2.14	1.6–480; 0.0024–2.41	Number of probes for each measuring	3
Cd	214.4	0.999	0.127	$1.27\cdot10^{-4}$–12	Plasma observation	Axial
Co	228.6	0.999	0.327	$3.27\cdot10^{-4}$–12		
Cr	283.5	0.999	0.435	$4.35\cdot10^{-4}$–12		
Cu	224.7; 324.7	0.999; 0.999	0.259	$9.07\cdot10^{-4}$–12; $2.59\cdot10^{-4}$–12		
Fe	259.9	0.999	0.118	$1.18\cdot10^{-4}$–12		
In	325.6	1.000	4	0.004–12		
K	404.7; 766.4	0.999; 0.999	0.378	0.798–300; $3.78\cdot10^{-4}$–1.2		
Li	323.2; 670.7	1.000; 0.999	$5.75\cdot10^{-2}$	0.079–12; $5.75\cdot10^{-5}$–1.2		
Mg	279.5; 285.2	0.999; 0.999	0.115	$1.15\cdot10^{-4}$–6.04; 4.03–120		
Mn	257.6	0.99992	$3.57\cdot10^{-2}$	$3.57\cdot10^{-5}$–12		
Na	330.2; 598.5	0.99994; 0.99989	4.75	7.98–480; 0.005–12		
Ni	231.6	0.99994	0.474	$4.74\cdot10^{-4}$–12		
P	214.9	1	1.59	0.002–120		
Pb	220.3	0.999	1.78	0.002–12		
Si	251.6	0.999	1.6	0.002–60		
Sr	407.7	0.999	$6.23\cdot10^{-3}$	$6.23\cdot10^{-6}$–2.41		
Tl	190.8	0.999	1.72	0.002–12		
Zn	213.8	0.999	$8.2\cdot10^{-2}$	$8.2\cdot10^{-5}$–12		
Hg	184.9	0.999	0. 192	$1.92\cdot10^{-4}$–12		

DPPH assay

Ethanolic solutions of extracts were prepared in a series of different concentrations (0.0098-2.5 mg/cm^3 for BPFEE; 0.039-2.5 mg/cm^3 for CPFEE). To 2.5 cm^3 of extract, 1 cm^3 of ethanol solution of DPPH radical ($3\cdot10^{-4}$ mol/dm^3) was added. The absorbance was measured on Cole Parmer Spectrophotometer at 517 nm after DPPH radical addition and after incubation at room temperature for 20 min (A_S). The absorbance was also determined for the ethanolic solution of DPPH radical (1 cm^3 DPPH radical solution with 2.5 cm^3 ethanol, A_C) and for the extracts without the DPPH radical (2.5 cm^3 extract with 1 cm^3 ethanol, A_B). The DPPH radical scavenging activity percentage (%) was calculated according to the formula (1) [22]:

$$\text{DPPH radical scavenging capacity (\%)} = 100 - \left[(A_S - A_B) \cdot \frac{100}{A_C} \right] \quad \text{.....(1)}$$

Butylated hydroxytoluene (BHT) was used as the positive control. All experiments were performed in triplicate.

Antimicrobial activity

The antimicrobial activity was determined by the microdilution method [23] against the reference and isolate strains of *Bacillus cereus, Salmonella enterica* and *Candida albicans*. The extracts were tested in the concentration range of 0.02-50 mg/cm^3. The serial dilution method was used to determine the minimal inhibitory concentration (MIC) in 96-well microtiter plates. Miller Hinton agar (Muller Hinton agar, MHA) was used for growing the bacterial strains for 18 hours at 37 °C, while Sabouraud Dextrose agar (SDA) was used to grow the yeast. The cell suspension was made in sterile Miller Hinton Broth (MHB) and Sabouraud Dextrose Broth (SDB). Using a densitometer (DEN-1 McFarland Densitometer, Biosan), the cell turbidity was adjusted to 0.5 McFarland (turbidity equivalent to a bacterial concentration of 10^8 cells/cm^3). The final inoculum density was 10^6 CFU/cm^3 (colony forming units). DMSO was used to create stocks of extract's solutions, which were then diluted in sterile MHB. After

testing the samples at serial dilutions (dilution factor 2x), the medium's final concentration range was 0.02–50 mg/cm^3. Following the preparation of the extracts' serial dilutions, the inoculum was added to the wells, and the plates were incubated at 37 °C for 24 hours.

Previously, the highest DMSO concentration of 5% was shown to have no effect on cell viability. As positive controls for bacteria and yeast were used chloramphenicol and nystatin, respectively. An inoculated well with medium without an agent was also left to test its sterility. Bacterial growth was observed by adding 20 µl of 0.5% aqueous TTC and incubating the plates for 30 minutes. By the following process, the minimum microbicidal concentration (MMC) was determined: Each well that showed no signs of growth had 10 µl of broth seeded on nutrient/SDA plates, and the plates were then incubated at 37 °C for 24 hours.

All experiments were carried out in three replicates.

Statistical analysis

All results were reported as mean value ± standard deviation from three measurements. Statistical comparisons were carried out by one-way ANOVA (analysis of variance) followed by Tukey's multiple comparison test by software SPSS 23.0 (IBM, USA). Differences are reflected as significant at $p < 0.05$.

Results and discussion

Phytochemical analysis of ethanolic extracts

TEM, TPC, TFC and concentration of elements of extracts are presented in Table 2.

Table 2. TEM, TPC, TFC and concentration of elements of BPFEE and CPFEE[a]

Sample	TEM, g/100g p.m.	TPC, mgGAE/g d.e.	TFC, mgRE/g d.e.	Concentration of elements, mg/dm^3
BPFEE	9.8 ± 0.1	89.8 ± 1.21	17.2 ± 0.18	Na (123), K (113.6), Ca (31.85), P (20.2), Zn (4.55), Mg (2.9), Fe (2.8), Ba (1.4), Sr (0.8), Pb (0.45), Cu (0.35), Li (0.25)
CPFEE	12 ± 0.1	75.6 ± 1.18	4.5 ± 0.2	Na (121.5), K (32), Ca (27.45), P (14.2), Fe (4.85), Ba (2.35), Pb (2.2), Mg (1.55), Zn (3.2), Sr (0.95), Cu (0.35), Li (0.25)

[a]Results are mean values of triplicate determination ± standard deviation.

CPFEE had 22.4% higher TEM yield than BPFEE; however, BPFEE showed almost 19% higher TPC and 4 times higher TFC than those for CPFEE. The obtained TEM yields for both extracts are in the range of those reported in the literature; 3.56-10.8% for BPFEE [19,24,25] and 4.35-18% for CPFEE [19,26,27]. In regards to the reported range of TPC values of medicinal and culinary plants [28], both peppers used in this study are considered as plants abundant in phenolic compounds. Our BPFEE showed higher TPC than those reported in the literature [24,29]. Interestingly, according to Nahak and Sahu [19] CPFEE contained twice as higher TPC than BPFEE, which is not the case in our study. These differences in both TEM and TPC values can be ascribed to several factors: the quality and maturity of the plant material, quantity of solvent, extraction technique and its applied conditions [30,31].

There are no literature data where TFC for BPFEE and CPFEE are expressed as the equivalent of rutine; only Akbar et al. [32] reported TFC value for BPFEE of 110.5±0.73 mgQCE/g of total flavonoids (quercentin equivalent). Although various phytochemical screenings confirmed the presence of flavonoids in CPFEE [19,33,34] literature data regarding flavonoid quantification in this extract is lacking.

The presence of 12 elements was detected in both extracts (Table 2). Among the macroelements, the highest presence of Na was detected in both extracts in similar amount. BPFEE contained higher amounts of K, Ca, P and Mg. Of the microelements, Cu and Li were detected in both extracts in similar amount. However, BPFEE contained higher amounts of Zn, but lower amount of Fe, compared to CPFEE. The presence of Mn, Cr, Ni, Cd, Ag, B and Co was not undetected, *i.e*. their contents were below the detection limit.

Antioxidative activity

EC_{50} of ethanolic extracts are presented in Table 3, while DPPH radical scavenging activity of extracts is shown in Figure 1. Statistical analysis of the results indicated significant difference between EC_{50} values for both extracts and BHT used as positive control.

Table 3. EC_{50} values of BPFEE and CPFEE[a]

Sample	EC_{50} at different incubation time, mg/cm^3	
	0 min	20 min
BPFEE	0.551 ± 0.02 b	0.104 ± 0.01 b
CPFEE	3.327 ± 0.02 c	0.378 ± 0.02 c
BHT	0.027 ± 0.001 a	0.021 ± 0.001 a

[a]Results are mean values of triplicate determination ± standard deviation. Column values (different small letters) are significantly different ($p < 0.05$) by Tukey's multiple range test.

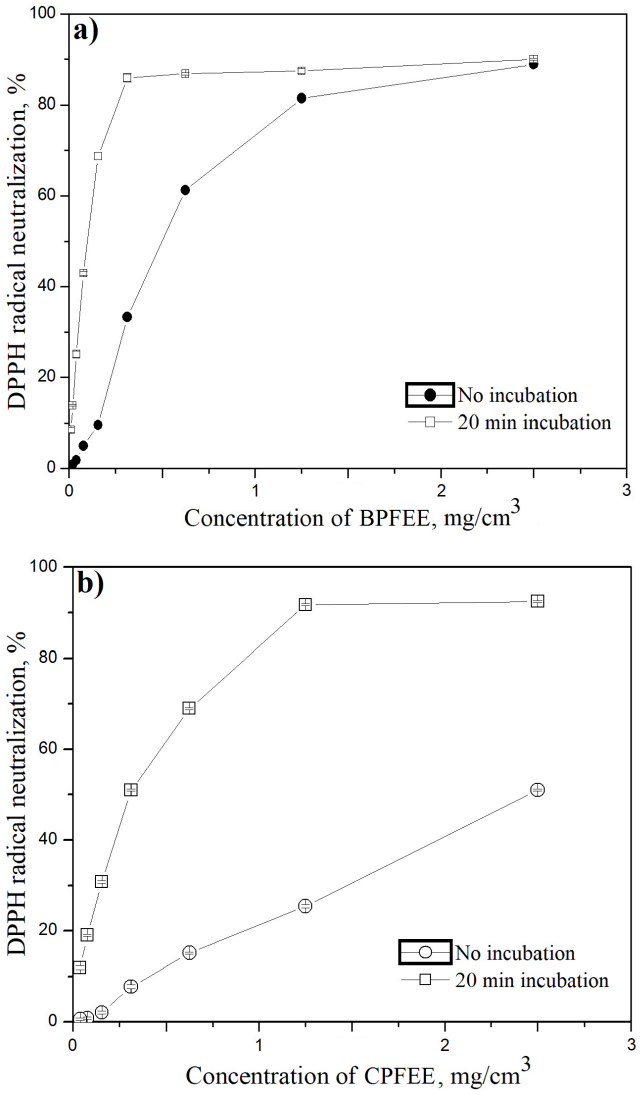

Figure 1. Degree of neutralization of DPPH radical of BPFEE (a) and CPFEE (b)

Based on the EC_{50} values, BPFEE showed better antioxidant activity than CPFEE. BPFEE exhibited significantly higher antioxidant activity than CPFEE at both 0 and 20 minutes of incubation, with its EC_{50} values decreasing substantially with longer incubation times. As a result, extended incubation enhances the antioxidant potential of both extracts, with BPFEE consistently showing better antioxidant activity. These results agree with the differences in TPC and TFC values of the analyzed extracts. A linear correlation between TPC and TFC and the antioxidant activity of plant species was established, since phenols and flavonoids possess the ability to "capture" free radicals [35].

According to Gülçin [24] aqueous and ethanolic extracts of black pepper showed similar DPPH scavenging activities due to their phenolic compounds; however, the interaction of these components has not been detailed. Akbar et al. [32] attributed better antioxidant activity by the DPPH assay of ethanolic extract of black pepper than its aqueous extract to almost twice higher content of flavonoids in the ethanolic extract, as well as the synergistic effect between the phenolic compounds and other phytochemicals extracted with ethanol. Nahak and Sahu [19] reported that ethanolic extracts of black and cubeb pepper showed the best DPPH radical scavenging activity, followed by their aqueous and then methanolic extracts; higher antioxidant activity of ethanolic extract of cubeb pepper than that for ethanolic extract of black pepper was correlated with its higher TPC. Khalaf et al. [36] noticed methanolic extract of cubeb pepper showed better antioxidant activity than extract from black pepper.

Generally, the solvent most used for polar flavonoids' extraction is a mixture of water and organic solvents, mostly methanol [37]. Since ethanol has lower dielectric constant of pure water (25.3 and 80.1 at 20 °C, respectively) [38] it will be more efficient for extracting bioactive polyphenolics. The antioxidant activity of both ethanolic extracts does not originate only from phenolic compounds, but is probably a consequence of the synergistic action of these compounds with other isolated biomolecules. Since the amount of detected phenolic compounds in this paper is not negligible, the focus of further research should be on the isolation and identification of phenolic compounds in produced extracts.

Antimicrobial activity

The results regarding the antimicrobial activities are shown in Table 4.

Table 4. MIC and MMC values for BPFEE and CPFEE[a]

Microorganism	MIC (mg/cm³)				MMC (mg/cm³)	
	BPFEE	CPFEE	Chloramphenicol	Nystatin	BPFEE	CPFEE
B. cereus food isolate	1.56 bB	3.12 cD	0.001 aA	Not treated	/ C	/ D
B. cereus ATCC 11778	0.78 cA	0.39 bA	0.0008 aA	Not treated	/ C	/ D
Salmonella spp. fecal isolate	6.25 cC	0.78 bB	0.006 aC	Not treated	/ C	/ D
S. enterica ssp. enterica ATCC 13078	6.25 cC	3.12 bD	0.003 aB	Not treated	25 A	25 C
C. albicans fecal isolate	1.56 bB	1.56 bC	Not treated	0.004 aA	50 B	6.25 B
C. albicans ATCC 24433	12.5 cD	1.56 bC	Not treated	0.004 aA	50 B	3.25 A

[a]Column (different capital letters) and row values (different small letters) are significantly different ($p < 0.05$) by Tukey's multiple range test.

Statistical analysis indicated significant difference between MIC values for both extracts and antimicrobial agents used as positive controls. Both extracts showed lower activity than the reference antimicrobial agents. Among the tested microorganisms, the most sensitive to both extracts was *B. cereus* ATCC 11778. CPFEE was more efficient inhibiting agent than BPFEE against *B. cereus* ATCC 11778, followed by *Salmonella* spp. fecal isolate, *C. albicans* ATCC 24433 and *S. enterica* ATCC 13078. *C. albicans* fecal isolate was equally susceptible to both extracts, while BPFEE only inhibited *B. cereus* food isolate slightly better than CPFEE. According to MMC values, CPFEE was much better anticandidal agent against both *C. albicans* strains, while both extracts were equally antibacterial against *S. enterica* ATCC 13078. However, both extracts failed to express

bactericidal effect against both *B. cereus* strains and *Salmonella* spp. fecal isolate.

Ethanolic extracts of black pepper were previously reported effective against *B. cereus* and *C. albicans* [39], while identical MIC values of 25 mg/cm^3 against *C. albicans* were reported for ethanolic extracts of black [40] and cubeb pepper [41]. Ethanolic cubeb pepper extract was more potent antimicrobial agent than its aqueous extracts since ethanol enables more efficient extraction of phytochemicals with antimicrobial properties [33] such as alkaloids, lignans, phenols, flavonoids etc.

The assumed antimicrobial mechanism of extracts includes interaction of extracted bioactive compounds with proteins constituting the microbial membrane, causing disruption in membrane's permeability and several scenarios: inhibition of functional enzymes, reduced mycelial germination or the lysis of cellular wall [42-44].

Conclusion

BPFEE and CPFEE were prepared by reflux extraction under identical conditions. Although CPFEE had higher TEM yield, BPFEE showed higher TPC and TFC values. The highest presence of Na was detected in both extracts in similar amount, while BPFEE contained higher amounts of K, Ca, P, Mg and Zn, but lower amount of Fe. Although BPFEE showed better antioxidant activity, CPFEE was more efficient microbial inhibiting agent and better anticandidal agent against both *C. albicans* strains. Both extracts were equally antibacterial against *S. enterica* ATCC 13078. These results indicate the potential of obtained extracts as effective natural antimicrobial and antioxidative additives in different pharmaceutical and cosmetic formulations, food products and dietary supplements. Since CPFEE is insufficiently investigated in the literature, this study also reflects the need for further research regarding detailed identification of isolated compounds and mechanisms responsible for antimicrobial and antioxidant activities. Although these peppers are only imported in Serbia, making their use in larger quantities non-economical, the waste plant material (after production of extracts) can be recycled and revalorized as biomass precursors for production of energy, bio-ethanol, biological derivatives and as additive in animal feed, maximizing the profit and enabling successful industrialization of the technological process.

Abbreviations: BHT, butylated hydroxytoluene; BPFEE, black pepper fruits' ethanolic extract; CPFEE, cubeb pepper fruits' ethanolic extract; DMSO, dimethylsulfoxide; DPPH, 1,1-diphenyl-2-picrylhydrazil; ICP-OES, inductively coupled plasma - optical emission spectrometry; MIC, minimum inhibitory concentration; MMC, minimum microbicidal concentration; TEM, total extractive matter; TFC, total flavonoids content; TPC, total phenolic content; TTC, triphenyltetrazolium chloride.

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Izvod

POREĐENJE FITOHEMIJSKIH SVOJSTAVA, ANTIMIKROBNE I ANTIOKSIDATIVNE AKTIVNOSTI ETANOLNIH EKSTRAKATA IZ PLODOVA CRNOG (*PIPER NIGRUM* L.) I KUBEBA BIBERA (*PIPER CUBEBA* L.) DOSTUPNIH U SRBIJI

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Upoređena su fitohemijska svojstva (ukupni sadržaj ekstraktivnih materija, fenola, flavonoida i minerala), antimikrobna i antioksidativna aktivnost etanolnih ekstrakata plodova crnog bibera (EEPCB) i etanolnog ekstrakta plodova kubeba bibera (EEPKB). Ekstrakti su dobijeni refluksom na temperaturi ključanja pri identičnim uslovima (solvomodul 1:10 m/v, vreme ekstrakcije: 120 min). EEPCB je imao veći sadržaj fenola i flavonoida, niži sadržaj ukupnih ekstraktivnih materija i bolju antioksidativnu aktivnost od EEPKB. Od makroelemenata, najveće prisustvo Na detektovano je u oba ekstrakta u sličnoj količini. EEPCB je sadržao veće količine K, Ca, P i Mg. Od mikroelemenata, Cu i Li su detektovani u oba ekstrakta u sličnoj količini. Međutim, EEPCB je sadržao veće količine Zn, ali nižu količinu Fe u poređenju sa EEPKB. Antimikrobna aktivnost je testirana protiv *Bacillus cereus*, *Salmonella* spp. i *Candida albicans*, referentnih sojeva i izolata. EEPKB je bio bolji bakteriostatski i antikandidalni agens. Ovi rezultati su dragoceni u promovisanju daljeg razvoja industrije prerade aromatičnog bilja, farmaceutske i prehrambene industrije.

Ključne reči: Antimikrobno; Crni biber (*Piper nigrum* L.); Kubeb biber (*Piper cubeba* L.); Ekstrakti; Sadržaj minerala; izolovanje; fenolna jedinjenja; Ekstrakcija po Soksletu